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REVIEW ARTICLE

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The Phaff school of yeast ecology

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Abstract Herman Jan Phaff's legacy includes pioneering work on the yeast cell envelope and the application of molecular approaches to yeast systematics. Clearly, his interest and knowledge spanned the whole gamut of yeast biology. Yet, his most original and most heartfelt contribution was to our understanding of the position occupied by yeasts in nature. This view developed through the juxtaposition of his childhood exposure to industrial fermentations and his training in the tradition of Beijerinck's Delft School of Microbiology. Through some of Phaff's recent writings, I have attempted to formulate the themes or principles that were implicit to his ecological thinking. Six focal points emerge. (1) Yeasts in themselves are a sufficient object of study. (2) A clear idea of a yeast community cannot be obtained unless the yeast species are correctly identified. (3) Ecologically meaningful conclusions require an adequate sample size. (4) The bacteriological dictum "everything is everywhere" is a poor account of yeast distributions. (5) The habitat is the cornerstone of yeast ecology. (6) Ecology is the most exciting aspect of yeast biology.

Keywords Yeast ecology · Yeast habitats · Herman J. Phaff (1913–2001)

Introduction: The Delft School of microbiology

As one reflects on the origins of modern microbiology, the names Louis Pasteur and Robert Koch immediately come to mind. But surprisingly, a clear genealogy linking these pioneers directly to noteworthy contemporaneous microbiologists is wanting. This is in dramatic contrast with the somewhat less ubiquitous pioneer, Martinus Beijerinck, discoverer of plant viruses and microbial sulfate reduction and major contributor to the idea of nitrogen fixation [4], as well as the first to isolate *Saccharomyces cerevisiae* from a natural source [10]. Pasteur and Koch focused principally on human disease and should probably be held accountable for the perennial juxtaposition, in academic politics, of microbiology, immunology, and pathology. The fact that microbiology succeeded in developing independently as a branch of biology distinct from medicine is due largely to Beijerinck and his followers. Appointed in 1895 to the Delft Polytechnic School, Beijerinck eventually founded the Delft School of Microbiology (a term later coined by van Niel) [4]. Beijerinck is the headwater of the world's *Who's Who* of Microbial Ecology, the beginning of a lineage that led eventually to the Phaff School of Yeast Ecology.

Beijerinck's closest collaborator, van Iterson, educated many microbiologists, including Albert Jan Kluyver, who in 1921 replaced Beijerinck in the Chair of Microbiology at Delft [4]. Kluyver was a prolific mentor. Among the many who studied under his guidance figure such names as van Niel, Wikén, Lodder, van der Walt, Starkey, Volcani, De Ley, Schlegel, Senez, and of course, Herman Jan Phaff. The fact that Beijerinck had a passion for plants (which at that time included microorganisms), combined with a profound dislike of medical microbiology and its students, insured that these talented individuals continued to educate others in the biology of microorganisms. The third and fourth generations of his intellectual descendants constitute a roster that is much too long to enumerate here. But the achievements of the individuals listed above and the historical context within which they were fostered serve to establish the foundation of a first element in the Credo of the Phaff School of Yeast Ecology, the beginning of a lineage that led eventually to the Phaff School of Yeast Ecology.

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Yeasts in themselves are a sufficient object of study

This position must continue to be defended at present, not only from the scoff of the omnipotent medical elite, but also from the neglect of many students of 'yeast' (without the 's'). Our reaction as enthusiasts of yeasts (with an 's') must go beyond mere indignation. We must have the conviction and the courage to pursue, as Herman Phaff did, the study of yeast biology 'up front' and not disguised so as to receive the sanction of any establishment, be it medical, industrial, or otherwise.

"[I] decided to concentrate my research efforts in the broad area of yeasts and to dissociate myself from the applied research pertaining to dried fruits [11]."

Phaff's fascination for yeasts had taken root in his family's fruit wine business. His dedication to ecology arose of a later epiphany.

"Early research dealt with yeasts involved in various aspects of food spoilage and food fermentations (...). My work then went in an ecological direction [12]."

In the tradition of Beijerinck and his disciples, Phaff never questioned the idea that yeast ecology is a topic worthy of pursuit for its own sake. As an ecologist, he was guided by a boundless instinct. He was never a schemer or a theoretician. I doubt very much that he one day decided first to examine aspects of the fundamental niche of yeasts, and later their communities. He knew all facts yeast-worthy and surrounded himself with individuals who were willing to explore the unknown. And he had the clairvoyance of encouraging them to do so.

In the earliest ecological work carried out in Phaff's laboratory, two of his students elucidated the physiological bases for the narrow association between a strange yeast and the gut of coprophagic rodents [1, 20]. He left it to others to argue over whether the yeast in question should be called *Saccharomycopsis guttulatus* or *Cyniclomyces guttulata*. Phaff's attention was rarely distracted by trivial issues.

During the same period, an interesting association was detected between certain yeasts and the gut of insects [19]. The general topic of yeast–insect associations was very broad and very complex, and Phaff recognized the need to associate with biologists who understood insects, yeasts, and their common habitats. His collaborations with Dobzhansky [6], Carson [2], Heed, and Starmer [23] led to thorough explorations of several such systems. The full impact of the cactus–yeast-*Drosophila* project will be realized when the expression 'fruit flies' is abandoned in favor of 'yeast flies'.

A clear idea (of a yeast community) cannot be obtained unless the yeast species are correctly identified

This second principle of the Phaff School is a quote from a review paper on yeast ecology [13]. There is more to this statement than meets the eye. First, it implies a sound basis for the delineation of species. Before the molecular revolution, which had its first impact on yeast systematics around 1970, species circumscription was more or less based (in modern parlance) on the concept of the autapomorphic morphospecies. Each name corresponded to a unique physiological profile, defined by a string of responses to the various growth tests proposed by Wickerham in 1951 [24]. The breadth of the species as defined physiologically was occasionally validated by mating experiments, but no other tools were available to assess gene-pool discontinuities.

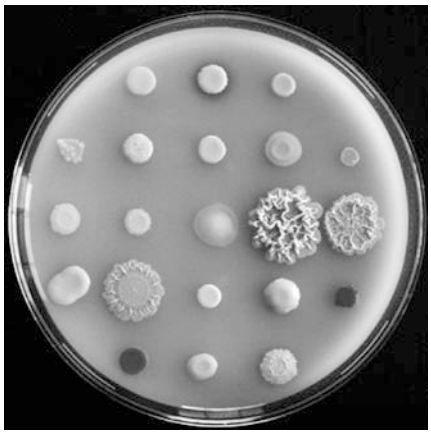

Fig. 1 A replica plate used in the nutritional characterization of large numbers of yeast isolates

At the time Phaff's ecological studies began, growth responses were determined individually in culture tubes with a vast array of liquid media. This approach, still in use in many laboratories, makes an ecological foray of sizeable proportions unthinkable. Phaff's adoption of a mechanized replica-plating system [21] changed this (Fig. 1). He once mentioned to me that the replica-plating technique allows one "to see ecological interactions." The depth of that statement only became clear to me several years later. The mere act of seeing yeast colonies growing side by side on the surface of an agar medium provides a *gestalt* that is difficult to explain. More practically, one obtains a better appreciation for community interactions from noticing the asymmetrical development of colonies on the diffusing hydrolysates of certain disaccharides, or the latent growth of methylotrophic yeasts on plates originally containing methylglucosides or acetone. Indeed, natural communities are seldom made up of axenic cultures. The term 'biocomplexity' is now used to acknowledge the existence of mixed, physiologically complementary species.

Species identification implies that we know what a species is. Phaff had a profoundly intuitive view on this matter. As the assessment of conspecificity by mating is restricted to the few known heterothallic species, the advent of molecular biology made it possible to verify genetic boundaries at the level of variation amongst genomes. In the late 1960s, Phaff's laboratory adopted analytical ultracentrifugation to determine molar guanine and cytosine richness of DNA [8].

"A new isolate which keys to (a certain) species is rarely identical and may vary in one or as many as seven or eight properties listed in the standard description of that particular species. The investigator is then faced with the dilemma of whether or not his isolate or isolates constitute a new species or whether it falls within the normal limits of strain variation of an already described species. The problem may be partially resolved by including among the routine tests a determination of the base composition of the nuclear DNA of such a species [15]."

Yeasts with significantly different G + C ratios were considered to represent different species, and those with similar ratios were regarded as potential candidates for conspecificity. The latter were examined by DNA reassociation, which eventually grew into a powerful test of species boundaries [9].

The obvious limitation of base composition was the ambiguity of equal numbers. Uncertainties could be resolved with DNA reassociation, although that approach suffered from the need always to conduct pair-wise reactions, thereby generating comparative data. Another limitation was that one does not know what is being measured. I had my own squabbles with Herman on this topic. In drafts of joint papers, he would write, "these strains share 80% of their DNA sequences." I would point out that relative binding is a comparative measure of heteroduplex DNA formation and cannot be translated directly into a quantitative expression of overall sequence similarity. Herman had little time for this kind of theoretical debate. He was a pragmatist. In the 1990s, the determination of DNA sequences became technically feasible, and after an admirably brief period of skepticism, Phaff, then in his eighties, realized the importance of this approach and became a convert. He arrived at the clear and unchangeable conviction that rDNA sequencing was the next step forward, not to provide a new species definition, as some would want us to believe, but to assist us in the discovery of new species, their meaningful classification, and their rapid and accurate identification.

Ecologically meaningful conclusions require an adequate sample size

Microbiologists rarely burden their minds with random stratified sampling designs, power analyses, or χ^2 distributions. Phaff was no exception, but he understood intuitively that an ecological generalization could not be formulated on the basis of a single isolation. The demonstration that the yeast community found in breeding and feeding sites of *Drosophila* was distinct from that found in the crops of the flies was based on a comparison of 134 isolates from breeding sites [2] with 240 isolates from crops of the insects [14]. Few studies, even now, involve that many isolates.

Generalizations about the constancy of a yeast community in a habitat and, as a result, the specificity of the habitat must be based on sustained, repeated sampling, as exemplified by a 1-year study of a single elm flux [17].

Fig. 2 H.J. Phaff sampling yeasts from cactus necroses on the island of Great Inagua, Bahamas (1983)

Whereas our ability to draw generalizations from ecological surveys hinges on examining adequate numbers of samples, practicality dictates that, in compensation, only "one colony of each type of yeast [is] picked for purification [5]." The probability of missing a species because of convergent colony types is offset by replication at the level of the samples. Serial dilutions and plate counts are usually superfluous. Sampling interesting substrates is far more important than the exact procedure used in sampling (Fig. 2). These are important practical considerations. The truth can sometimes be hidden by an inordinate desire to reach it through stifling rules.

Phaff's parsimonious ecological principles spilled over into his taxonomic thinking, leading him to encourage "caution in the indiscriminate description of new species based on only one or a few strains [5]." The majority of yeast species described by Phaff were documented by multiple isolates. In some cases, the number exceeded one hundred [16].

The bacteriological dictum "everything is everywhere" is a poor account of yeast distributions

"Numerous surveys of substrates that were suspected to harbor yeasts (...) have revealed that yeasts appear to be far less ubiquitous than many bacterial species. Specialization for habitat appears to be the rule rather than the exception [13]."

I remember Herman showing students in his yeast course photographs of various yeast habitats: foaming

tree stumps, necrotic cacti, viscid slime fluxes. Envious of his gallivanting across the planet in search of suitable substrates, I felt cheated by having to sit in a lab sprinkling agar plates with frass collected (by him) in some enchanted forest, far away. But much worse, I was mortified by the thought that if someone were to ask me to go to the field and locate a genuine yeast community, I would not know where to start. How did he know? Like many who have experienced the pleasure of working with yeasts, I had not been sufficiently been impressed with the historical battle to convince the world that microbial life does not pop out of nothing [5], a battle that apparently has yet to be won. Yeasts, were they everywhere, as is often thought, would first have to grow somewhere and then get there. If yeasts grow on grape skins and in culture collections, as is often claimed, how do they get there?

Incidentally, ''Beijerinck's law'' is in fact attributable to Baas-Becking, a contemporary Danish microbiologist who was also the originator of the Gaia paradigm [3, 18], another attractive but simplistic metaphor.

The habitat is the cornerstone of yeast ecology

In a short review paper entitled ''Ecology of yeasts with actual and potential value in biotechnology'' [10], Phaff discussed the notion of habitat specificity. He explained the little that was known on the habitats and vectors of some industrially important species. In doing so, he revealed what he meant by ''Ecology'', namely, how yeasts interact with other organisms, but more importantly, where they live, and why they live there.

The life cycle and the physiological properties of a yeast species must play a fundamental role in determining its habitat specificity. However, the properties assessed in the laboratory are usually not sufficient to predict where a particular yeast species will be found in nature. In some cases, as Phaff pointed out [10], the relationship between habitat and physiology is obvious, at least in appearance. Methylotrophs abound in decaying plant tissue, the principal source of methanol in nature. This may seem trite and even misleading. Indeed some of the methylotrophic species listed in Phaff's review had been found only in seawater, soil, or creek water. Those particular species, of course, had been described from single isolates and were undoubtedly contaminants. Recent studies of yeast communities of slime fluxes of tropical trees [7, and unpublished data] demonstrated that decaying wood is a reproducible source of methylotrophs.

In other cases, one is at a loss to explain why a yeast species occurs where it does. In spite of numerous attempts to make grapes the natural habitat of *Saccharomyces cerevisiae* or even to deny that it has one, the best speculation is still that the wild forms are associated with an interface involving *Drosophila* spp. [14], oaks, and the surrounding soil [22, 25].

Fig. 3 H.J. Phaff yeast-hunting in Northern Argentina (1991)

Ecology is the most exciting aspect of yeast biology

''I developed a greater interest in yeasts found in nature. (...) My main research interest in microbiology ultimately developed into concern for ecological and resultant taxonomic questions [11].''

The intimate relationship between the ecologist and the yeast begins in the field (Fig. 3), where a potential habitat is first detected, examined, smelled, and sampled. The relationship grows further as one conducts ''yeast isolations in improvised field laboratories,'' including forest campsites, motels [11], or even a rolling, yawing, and pitching ship on a stormy sea. It takes on a new dimension as each isolate is examined under the microscope and characterized physiologically. The experience is finally crowned by the joy of documenting the *genuine discovery* of a new species, an emotion that will remain forever unknown to those who confine their activities to culture collections. The connection of a human with a yeast species reaches full maturity when, after careful analysis, the ecologist returns to the field and looks again at the original habitat with the newly acquired knowledge of its yeast species composition.

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